Australian Government
Department of Defence
Defence Science and
Technology Organisation

Biosecurity Management of Submarine Niche Areas: the Effect of Water Pressure on Biofouling Survival

Clare Grandison and Richard Piola

Maritime Division
Defence Science and Technology Organisation

DSTO-TR-2930

ABSTRACT

This study showed that exposure to pressure change has a minimal impact on the survival of common biofouling taxa; however, fouling taxa survival may be adversely impacted by declining local water quality. Survival was evident across all water pressure treatment regimes, although the 48 hour treatment (Trial 3: 130 kPa, 48 hours, 4 pressure cycles) resulted in decreased survival compared to both 24 hour treatments (Trial 1: 130 kPa, 24 hours, 2 pressure cycles and Trial 2: 200 kPa, 24 hours, 2 pressure cycles). Whilst the study resulted in some mortality of the fouling taxa, significant survival of the fouling taxa was still recorded, thus showing water pressure change to be insufficient as a control mechanism. Based on the results of the current study, a sole reliance on the effects of water pressure change during operational diving and surfacing manoeuvres is not recommended as a niche area fouling mitigation strategy for submarines and consideration of other mitigation strategies is required to ensure biofouling is controlled to reduce operational impacts and biosecurity risks.

RELEASE LIMITATION

Approved for public release

Published by

DSTO Defence Science and Technology Organisation
506 Lorimer St
Fishermans Bend, Victoria 3207 Australia

Telephone: 1300 333 362
Fax: (03) 9626 7999

AR-015-833
January 2014

APPROVED FOR PUBLIC RELEASE

Biosecurity Management of Submarine Niche Areas: the Effect of Water Pressure on Biofouling Survival

Executive Summary

Anecdotal reports received from retired submariners have suggested that common biofouling taxa occurring on Royal Australian Navy (RAN) boats may be adversely affected by the water pressure changes experienced during routine diving and surfacing of the boat. This may provide a mitigation strategy for biosecurity management of submarine niche areas.

The overall aim of the present study was to examine whether anecdotal reports of biofouling mortality, resulting from pressure changes experienced by submarines during normal diving and surfacing operations, were realistic and able to be replicated under experimental conditions. This report details the result of the experimental program developed to examine the effects of variable water pressures and water pressure exposure times to the survival of common biofouling organisms.

This study showed that exposure to pressure change has a minimal impact on the survival of common biofouling taxa. However, fouling taxa survival may be adversely impacted by declining local water quality. Survival was evident across all water pressure treatment regimes, although the 48 hour treatment (Trial 3: 130 kPa, 48 hours, 4 pressure cycles) resulted in decreased survival compared to both 24 hour treatments (Trial 1: 130 kPa, 24 hours, 2 pressure cycles and Trial 2: 200 kPa, 24 hours, 2 pressure cycles). Whilst the study resulted in some mortality of the fouling taxa, significant survival of the fouling taxa was still recorded, thus showing water pressure change to be insufficient as a control mechanism.

While the present study attempted to replicate the changes in water pressure that may be experienced by biofouling taxa in the free flood spaces of a submarine, it should be noted that they are not completely reflective of the conditions experience by biofouling in the free flood spaces of a submarine. Most submarine niches and free flood spaces would likely receive adequate free exchange of water such that poor local water quality conditions would not develop during the course of a voyage. Additionally, real world water pressure changes associated with a submarine diving and/or surfacing would also have corresponding changes in water temperature (e.g. decreasing water temperatures associated with depth). The possibility exists that a combination of rapid pressure and temperature changes (analogous to conditions experience when a

submarine dives to depth) may act synergistically, compounding the impact that either of these parameters have on biofouling survival when considered in isolation.

Based on the results of the current study, a sole reliance on the effects of water pressure change during operational diving and surfacing manoeuvres is not recommended as a niche area fouling mitigation strategy for the current Collins Class fleet, or the SEA 1000 Future Submarine. Consideration of other mitigation strategies is required to ensure biofouling is controlled to reduce operational impacts and biosecurity risks.

Authors

Clare Grandison
Maritime Division

Clare Grandison joined DSTO in 2009 after completing a BEnvSc(Hons) at RMIT University. She also holds a MSocSc (International Urban and Environmental Management). Since joining the Environmental Research and Biotechnology Group, she has been involved in research into marine pollution control and management (including ship board waste auditing), fouling prevention strategies for internal seawater systems and marine biosecurity management, as well as performing environmental and technological risk assessments for new acquisitions and fleet-in-being.

Dr Richard Piola
Maritime Division

Richard Piola joined the Environmental Research and Biotechnology Group in 2010. After completing his PhD in Marine Ecology and Ecotoxicology from the University of New South Wales in 2007, Richard worked for several years as a research scientist and consultant with the Cawthron Institute in New Zealand, specialising in the fields of marine biosecurity, vessel biofouling assessment and management, and the development of tools for the control and eradication of unwanted marine pests. His primary research interests at DSTO continue to be biofouling and marine pest management, biofouling control, and the improvement of biosecurity inspection and incursion response protocols.

This page is intentionally blank

Contents

This page is intentionally blank

1. Introduction

As the understanding of external hull biofouling and the risks associated with ballast water vectors becomes more mature and technologies to control fouling in these areas improve (ASA, 2007, Coutts and Dodgshun, 2007), the management of vessel-borne biosecurity risks is becoming increasingly focussed on biofouling associated with so-called 'niche' areas.

Vessel niche areas are defined as areas on a ship that may be more susceptible to biofouling relative to exposed hull surfaces. They are characterised by a number of factors that make them an increased biosecurity risk for the transport of non-indigenous species (NIS). Niche areas are typically exposed to external water, subject to different hydrodynamic forces, susceptible to coating system wear or damage, often difficult to access for inspection or to apply protective anti-fouling coatings, and require specialised biofouling management and mitigation strategies (IMO, 2011, URS, 2006). Military vessels often have more niches than commercial sector vessels due to the specialised nature of their operations and the tasks they are required to perform.

As awareness of the operational and biosecurity risks associated with niches increases, surface vessel niche areas are becoming better identified and are beginning to have fouling mitigation strategies tailored to their particular fouling challenges. However, submarine niche areas are less well understood and present a more complex array of niches due to their design and operational profile. In particular, submarine hulls have a number of external openings (known as 'free flood spaces') that freely exchange water with the surrounding environment, independent of the boat's ballast water capacity. Free flood spaces lie outside the pressure hull and are contained by the casing (light hull). They enable rapid draining and submersion of the superstructure as well as surrounding items (such as the anchor well and fin) that do not need to be maintained at pressure (Polglaze, 2009). The design, number and size of free flood spaces will vary from class-to-class and possibly boat-to-boat, dependent on the features of the particular vessel.

Figure 1 demonstrates the diversity of hull features on the Collins Class submarine that are recognised as potential niche fouling areas (URS, 2006). Free flood spaces exhibit all the characteristics of niche areas. As such, they are considered an area of risk for facilitating the translocation of NIS by colonisation with organisms taken up from the surrounding environment. These spaces provide shelter from turbulent flows, multiple attachment points and permanent water (to some degree) (URS, 2006). Free flood spaces may also be at risk of accumulating biofouling to such an extent that it may impact the rapid draining necessary for safe and effective operation of the boat. Importantly, many submarine free flood spaces may be inaccessible for inspection and biofouling mitigation. As such, it becomes imperative to consider options for the pro-active management of biofouling in these free flood spaces, as management by operational means may not be feasible.

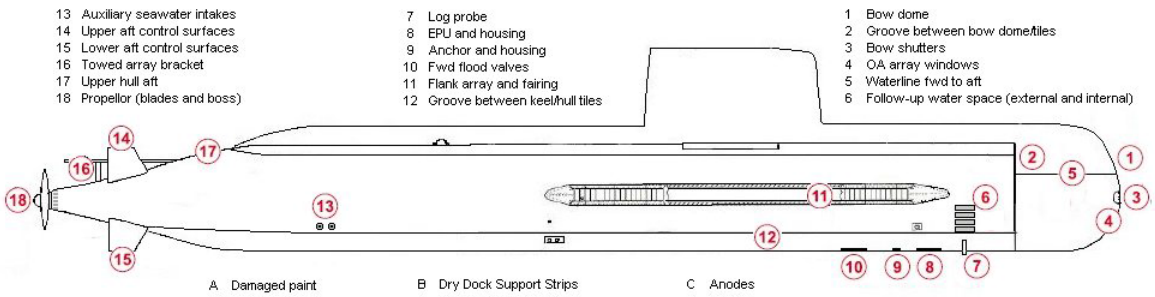

Figure 1. Collins Class submarine hull features (URS, 2006)

Submarine free flood spaces and the biofouling contained therein, are subjected to rapid pressure changes during the action of diving and surfacing of the boat. This has the potential to adversely affect the viability of the fouling organisms. It has been reported anecdotally by submariners that algal fouling occurring on the external submarine hulls was bleached during regular diving operations (J Taubman, pers. comm., September 2012).

The overall aim of the present study was to examine whether anecdotal reports of biofouling mortality resulting from pressure changes experienced by submarines during normal diving and surfacing operations were realistic and able to be replicated under experimental conditions. This report details the result of the experimental program developed to examine the effects of variable water pressures and water pressure exposure times, to the survival of common biofouling organisms. This experimental program was developed to investigate the impacts of pressure changes experienced during diving on common fouling organisms know to occur on RAN vessels (URS, 2006) for the purpose of developing biosecurity mitigation guidelines for submarine free flood spaces.

2. Materials and Methods

2.1 Water pressure test chamber

The effect of water pressure on biofouling organisms was assessed by placing a selection of common hull biofouling organisms in an underwater pressure tank. The pressure vessel used for all experiments was a purpose built Jahco Welding Engineers (now Britannia: Jahco, Melbourne, Australia) 2000 L cylindrical water pressure vessel (design registration number V358-93), with a certified working pressure/design pressure of 2000 kPa. This is equivalent to the pressure at a depth of approximately 200 m (Figure 2).

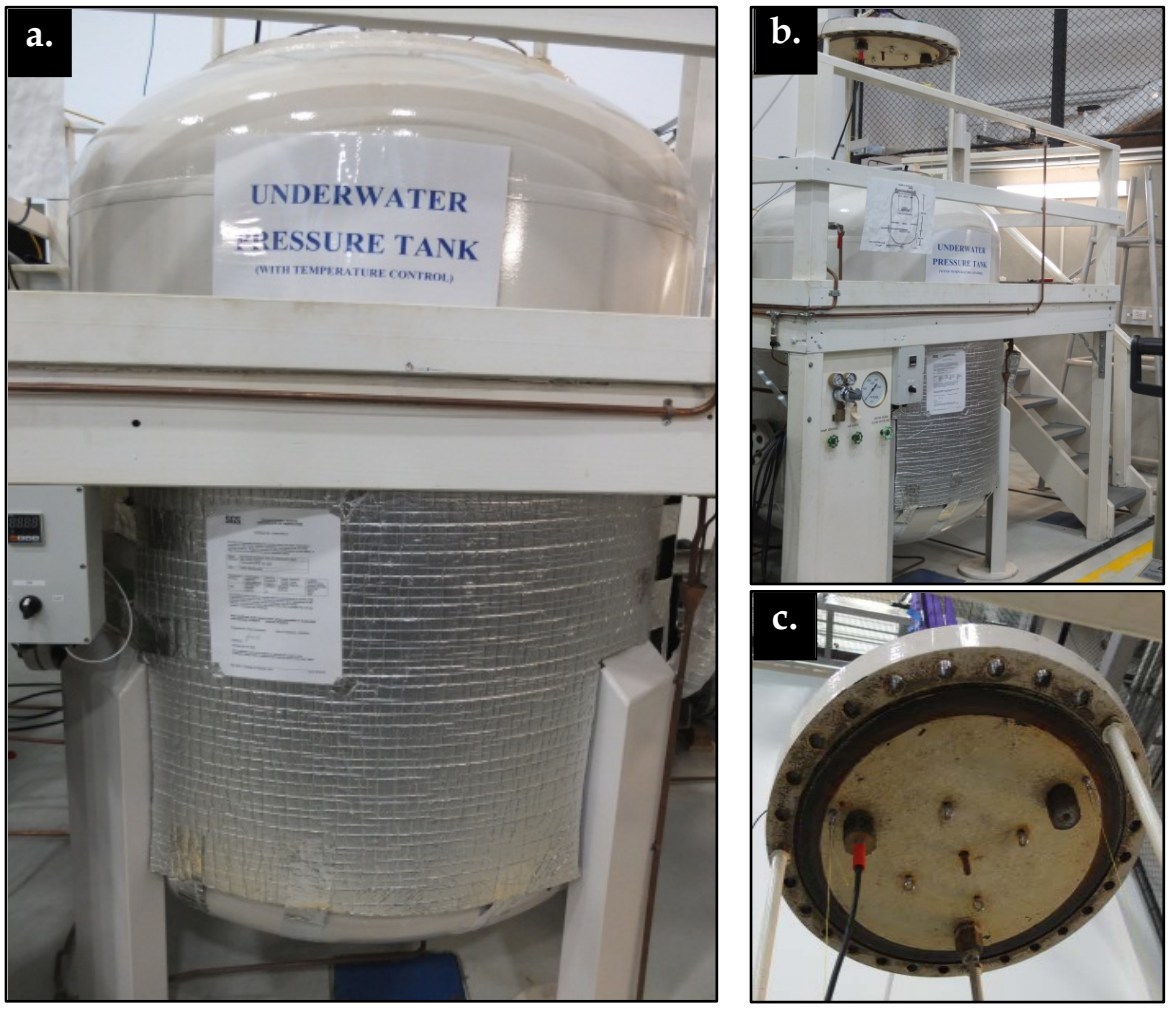

Figure 2. Photographs showing (a) the DSTO 2000L Jahco Water Pressure Vessel used during the study, (b) the pressure vessel, control panel and access platform, and (c) the underside of the pressure vessel lid showing attachment points for test samples.

2.2 Water pressure testing procedure

A representative diagram outlining the setup of the pressure testing procedure is shown in Figure 3. Given the risk of corrosion to the pressure vessel if filled with sea water, the tank was filled with freshwater and test organisms were isolated in experimental treatment units containing sea water for the duration of the pressurisation process. Experimental treatment units comprised two large polyethylene bags, one placed inside the other (i.e. 'double-bagged'), externally supported by a mesh bag (Figure 4a). The inner polyethylene bag was then filled with approximately 5 L of natural sea water and test organisms were placed inside. To seal, the top of the inner polyethylene bag was twisted tight to expel any air, then folded over and secured with electrical tape prior to tying off with two cable ties. This process was repeated for the outer bag.

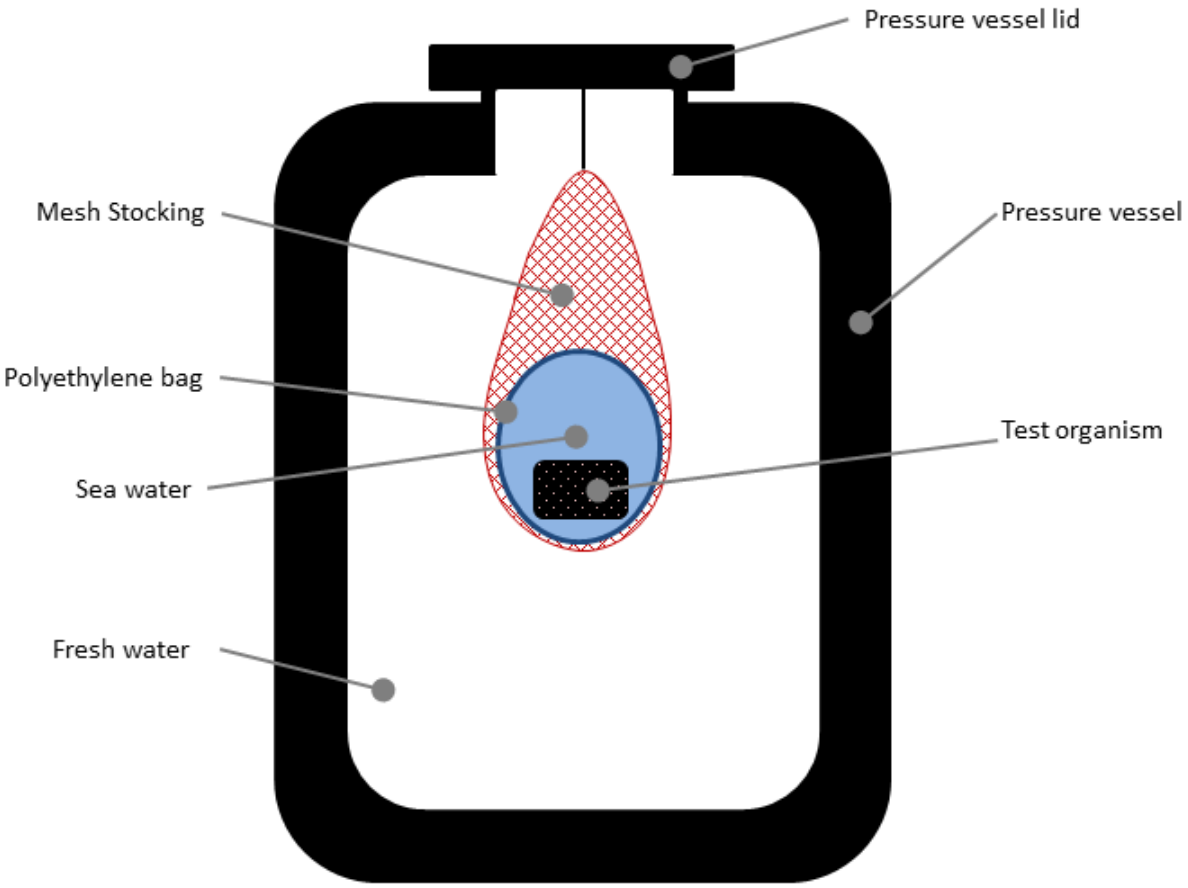

Figure 3. Diagrammatic representation of the experimental setup used to subject biofouling test organisms to a range of water pressures. The setup included a 2000 L pressure vessel and lid filled with freshwater, experimental treatment units comprised of polyethylene bags and mussel stocking mesh filled with sea water and holding the test organisms.

For testing, experimental treatment units were attached to the underside of the lid of the pressure vessel by tying the mesh through in-built eye hooks (Figure 4b). The experimental treatment units had a small weight attached to the bottom of the mesh to ensure the bags sat correctly when the lid was lowered and the chamber closed. The lid was then lowered onto the

chamber and secured into position. For Trial 1 (130 kPa, 24 h, 2 pressure cycles) a total of four experimental treatment units were placed in the chamber for each replicate treatment run, with each of the test organism/assemblages to be treated (i.e. mussels, barnacles, algae, settlement plate) in an individual bag. During Trials 2 (200 kPa, 24 h, 2 pressure cycles) and 3 (130 kPa, 48 h, 4 pressure cycles) a total of six experimental treatment units were placed into the chamber concurrently for each replicate treatment run. For Trials 2 and 3, each treatment bag contained a combination of large mussels and algae (in a single bag) or small mussels and the assemblage plate (in a single bag).

Control experimental treatment units were handled and processed in an identical manner to pressure treatments, except, rather than being placed in the pressure chamber, they were suspended in a freshwater-filled 240 L control tank (adjacent to the test chamber) at ambient pressure for the prescribed exposure period (Figure 4c). During the treatment period a lid was placed over the control tank to ensure the samples remained in darkness.

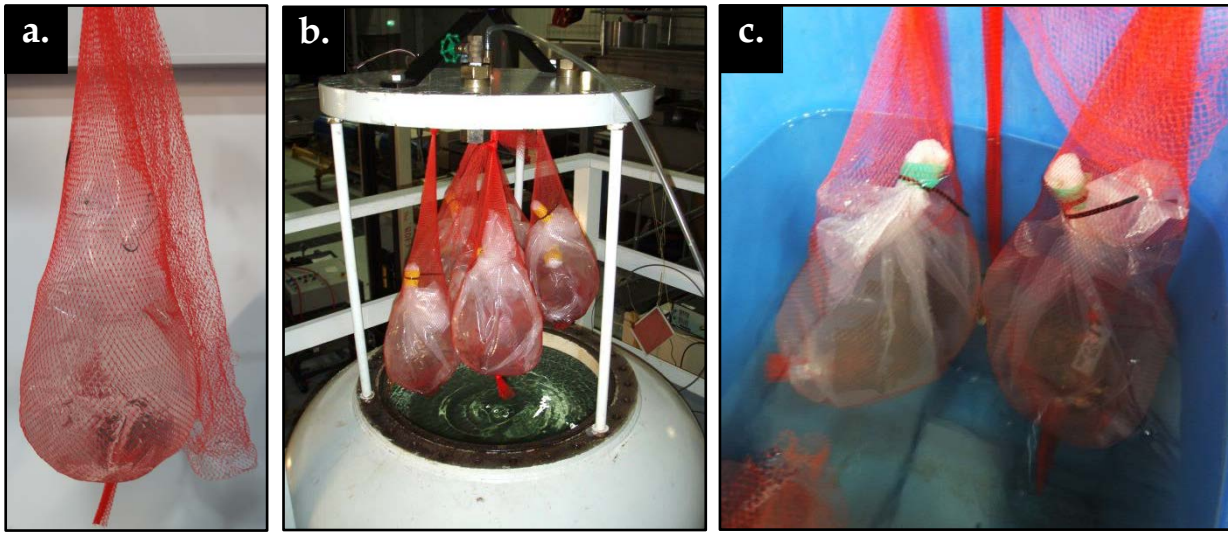

Figure 4. Photographs showing (a) an individual replicate experimental treatment unit comprising two polyethylene bags filled with sea water and supported by mesh, (b) replicate experimental treatment unit attached to the underside of the pressure vessel lid prior to testing, and (c) replicate control experimental treatment unit immersed in the control water tank.

2.3 Pressure treatment profiles

Treatment units were subjected to three different pressure treatment profiles (Table 1). Test organisms were subjected to two different water pressures, 130 kPa (Trial 1 and 3) and 200 kPa (Trial 2). The former pressure was selected as it lies within the conceivable operating depth range for a submarine undertaking routine operations, while the latter was selected because it is the maximum pressure attainable in the test pressure vessel used. During Trials 1 and 2, test organisms were subjected to the prescribed water pressures for a total of 24 h, over which time the pressure in the test chamber was cycled twice (simulating a dive, surface, dive, surface profile). Pressure cycling involved gradually reducing the pressure in the test chamber to zero (over a period of approximately 2 min) and then slowly increasing it again to the desired test

pressure. During pre- and post-pressure cycling, the pressure vessel remained sealed and the test samples remained immersed. During Trial 3, test organisms were subjected to the prescribed water pressures for a total of 48 h, over which time the pressure in the test chamber was cycled four times.

Table 1. (n = 3 replicates for each test organism per Trial)

Trial Number	Pressure (kPa)	Treatment time (h)	Number of pressure cycles	Cycle time (h)
Trial 1	130	24	2	12
Trial 2	200	24	2	12
Trial 3	130	48	4	12

2.4 Test organisms

Three discrete types of biofouling organisms (mussels, barnacles and algae) were subjected to testing in the pressure chamber. In addition, a mixed fouling assemblage grown *in situ* in Port Phillip Bay waters via natural recruitment and settlement was also included for testing. The organisms were selected to provide a representative sample of common biofouling taxa and included both calcareous and soft-bodied organisms. Each pressure treatment profile examination (i.e. Trial 1, 2 and 3; Table 1) involved treating three replicate units of the biofouling organisms in question (i.e. mussels, barnacles, whole assemblages, algae).

2.4.1 Mussels

Mytilus galloprovincialis planulatus were collected from deployed biofouled substrates and ropes at the DSTO Marine Coatings and Corrosion Test Facility, Williamstown, Melbourne. Small (~ 10-30 mm) and large (>50 mm) mussels were examined separately during the experiment. Each replicate treatment unit comprised of 10 mussels assessed as alive prior to immersion. Collected individuals were grouped in a mesh bag and held in the field for ~7 d to recover prior to treatment (Figure 5a).

2.4.2 Barnacles

Barnacles were collected from suitably fouled substrates at the DSTO Marine Coatings and Corrosion Test Facility, Williamstown, Melbourne. Substrates were selected to provide sufficient numbers of individuals to enable analysis of the impact of pressure (Figure 5b). Prior to testing, barnacles were observed for feeding activity (i.e. cirri movement) and/or movement response of the operculum when touched to indicate organism viability.

2.4.3 Mixed fouling assemblage

Each mixed fouling assemblage replicate unit comprised of one settlement plate (~100 x 50 mm) that had been allowed to accumulate a natural fouling assemblage (over approximately 6 months) at the DSTO Marine Coatings and Corrosion Test Facility, Williamstown, Melbourne.

Assemblages were assessed prior to treatment to identify and count the major fouling taxa present and determine organism viability. Fouling types recorded included colonial and solitary ascidians, arborescent and encrusting bryozoans, calcareous tubeworms and sponges (Figure 5c). Prior to testing, the mixed fouling assemblage plates were photographed to enable post-test comparison.

2.4.4 Algae

Each replicate unit comprised of approximately 2-4 small-sized plants of *Undaria pinnatifida* collected from submerged structures at the DSTO Marine Coatings and Corrosion Test Facility, Williamstown, Melbourne. Prior to testing, the algal fronds were photographed to enable post-test comparison (Figure 5d).

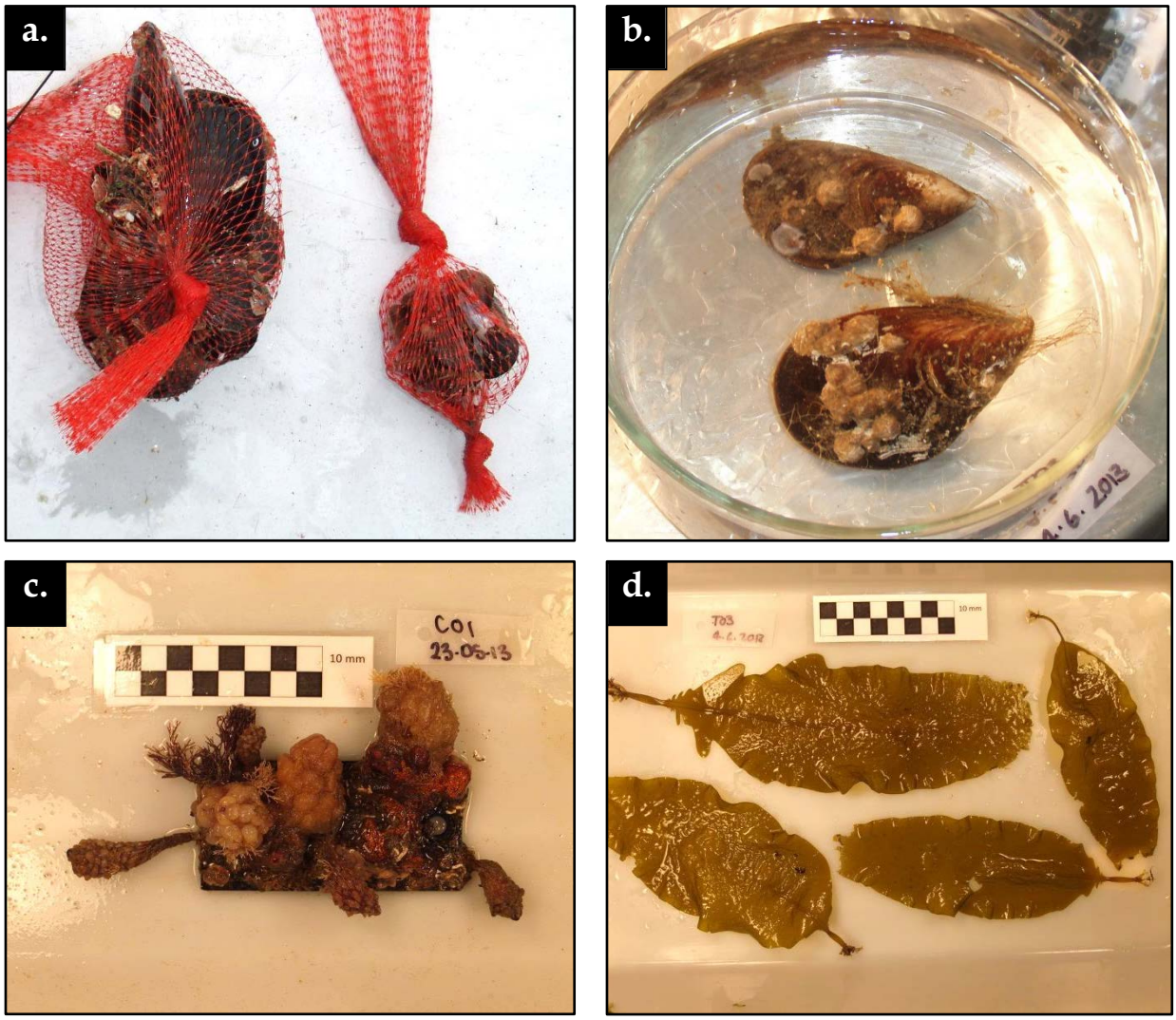

Figure 5. Photographs showing the replicate test organisms treatment units, including (a) large and small mussels in mesh bags, (b) barnacles (growing on a substratum of mussel shell), (c) a settlement plate containing a well-developed fouling assemblage, and (d) multiple algal (Undaria pinnatifida) plantlets

2.5 Post-treatment survival assessment

Upon completion of each pressure treatment run, exposed pressure treatment and control units were transported back into the field, where they were held in a flow through seawater tank awaiting post-treatment survival assessment. Survival assessments were conducted for each group of test organisms 7 days post-treatment. Samples were returned to the laboratory for assessment of survival.

The method for conducting post-treatment survival assessments of fouling taxa following pressure treatment trials differed based on the organisms in question. In some cases, pre- and post-pressure treatment photograph comparison was used to determine the extent of any

pressure related impacts. Survival was expressed as percentage survival, based on pre- and post-treatment numbers of live individuals recorded. Specific organism survival assessment procedures were:

- *Mussels*: Dead mussels were distinguished from live individuals by the presence of open empty shells, or partially open non-responsive shells with putrefied contents.
- *Barnacles*: Barnacles were classified as dead based on: (i) a lack of cirri movement during 10 min of observation, (ii) if cirri were unresponsively extended from the operculum, and/or (iii) if there was no movement response when touching the operculum (i.e. retraction of mantle or closing reflex of operculum plates).
- *Colonial ascidians*: Mortality was determined by a lack of colony growth over the post-treatment period (based on before and after photograph comparisons), discolouration and decomposition of tissues and/or complete absence of the colony.
- *Solitary ascidians*: Solitary ascidians were classified as dead if: (i) their siphons were closed, (ii) there was obvious tissue decomposition, and/or (iii) there was no response to touch stimuli.
- *Bryozoans*: Each colony was examined under a dissecting microscope for signs of lophophore movement – if no movement was observed, the organism was considered dead. Each colony was also examined to determine whether there was any tissue discolouration or decomposition.
- *Calcareous tubeworms*: The percentage survival of serpulids was determined by randomly removing 20 individual tubes from each settlement plate, and dissecting the tube under a microscope to determine if a live worm was present within.
- *Algae*: *U. pinnatifida* health and survival was assessed by measuring the change in surface area of plant fronds using digital photographs taken before and after treatment (0 and 7 d). Surface area measurements were made using ImageJ image analysis software (Rasband 1997-2008).

2.6 Water quality

Water quality was measured in the pressure treatment and control experimental treatment units just prior to and immediately following the immersion testing period. Water quality parameters measured included temperature (°C), salinity (ppt), dissolved oxygen (mg L^{-1}) and pH. All measurements were made using a YSI 6920 V2 Compact sonde.

2.7 Data analysis and interpretation

Analysis of variance (ANOVA) was used to compare the difference in percentage survival of small and large mussels, barnacles, calcareous tubeworms and whole assemblage taxa, with the factors of pressure profile (130 kPa [24 h], 200 kPa [24 h], 130 kPa [48 h]) and treatment group (pressure treatment, control) being examined. For whole assemblages, examining the effects of each treatment was complicated by the fact that the settlement plates contained an inconsistent variety of taxa in different abundances. Therefore, analysis was based on the percentage survival

of all organisms recorded within the assemblage pre- and post-treatment (i.e. the total number of individuals). ANOVA was also used to compare percentage change in surface area of algal fronds. Tukey's post-hoc comparisons were performed to test for difference among individual pressure treatment profiles. All data were assessed for homogeneity of variance and normality using Levene's test and residuals frequency histograms, respectively. All ANOVA were conducted using SPSS Version 19 (IBM Corp., Armonk, NY, USA).

3. Results

3.1 Physical parameters

The mean water temperature, salinity, dissolved oxygen (DO) and pH values recorded in the experimental units containing each taxa (large mussels, small mussels, whole assemblages, algae) during Trials 1 (130 kPa, 24 h, 2 pressure cycles), 2 (200 kPa, 24 h, 2 pressure cycles) and 3 (130 kPa, 48 h, 4 pressure cycles) are presented in Table 2.

Temperatures recorded during Trial 1 (16.7 – 17.3 ^{o}C) were ~ 2 ^{o}C warmer than those recorded during Trials 2 and 3 (14.9 – 15.4 ^{o}C and 14.8 – 15.4 ^{o}C, respectively). Temperatures recorded at the end of each trial period showed little fluctuation, with mean temperatures increasing or decreasing by < 1 ^{o}C (Table 2).

Salinity values recorded during Trials 1 and 2 (34.6 – 35.1 ‰ and 35.7 – 35.6 ‰, respectively) were approximately 5 – 7 ‰ higher than those recorded during Trial 3 (29.1 – 30.7 ‰). This was due to lowered salinity in sea water collected from Williamstown used in Trial 3, as a result of a recent rainfall event. Similarly to patterns observed for temperature, there was virtually no change in salinity values in experimental units recorded at the start and end of the exposure period (Table 2).

The observed percentage decrease in the DO measured in experimental units over the duration of each trial exposure period (i.e. 24 or 48 h) are presented for each taxa in Figure 6. Overall, percentage decrease in DO was less in Trial 1 (130 kPa, 24 h) relative to Trial 2 (200 kPa, 24 h) and 3 (130 kPa, 48 h); however, the decrease in DO in Trial 1 bags containing large mussels was considerably greater that in bags holding small mussels, whole assemblages and algae (Figure 6). The percentage decrease in DO in Trial 2 bags (sealed for 24 h) was similar to that in Trial 3 bags (sealed for 48 h). In general, the percentage decrease in DO recorded in corresponding treatment and control units was similar for each taxon in each trial.

Table 2. Summary of physico-chemical data (temperature, salinity, dissolved oxygen and pH) recorded within the experimental treatment units of different biofouling test organisms immediately before and after pressure testing. Values represent means ±1SE (n= 3).

Pressure Profile, Taxa, Treatment Group	Temperature (°C)		Salinity (‰)		DO (mg L^{-1})		pH	
	Start	End	Start	End	Start	End	Start	End
(a) 130 kPa (24 h)								
Mussels (Large)								
Treatment	16.7 ± 0.8	16.4 ± 0.3	34.8 ± 0.7	34.9 ± 0.6	7.4 ± 0.4	2.1 ± 0.2	7.86 ± 0.1	7.32 ± 0.0
Control	16.8 ± 0.8	16.6 ± 0.3	34.7 ± 0.7	34.7 ± 0.7	7.39 ± 0.4	2.60 ± 0.4	7.88 ± 0.1	7.29 ± 0.1
Mussels (Small)								
Treatment	16.7 ± 0.8	16.4 ± 0.3	34.6 ± 0.9	34.6 ± 0.6	7.37 ± 0.4	4.64 ± 0.3	7.85 ± 0.1	7.61 ± 0.0
Control	16.8 ± 0.8	16.6 ± 0.3	34.8 ± 0.7	34.9 ± 0.7	7.39 ± 0.4	4.65 ± 0.4	7.87 ± 0.1	7.57 ± 0.1
Settlement plates								
Treatment	17.3 ± 0.8	16.5 ± 0.2	35.1 ± 0.5	34.9 ± 0.4	7.2 ± 0.3	4.38 ± 0.6	7.8 ± 0.1	7.62 ± 0.0
Control	17.2 ± 0.8	16.6 ± 0.3	35.0 ± 0.5	35.0 ± 0.5	7.18 ± 0.3	3.92 ± 0.5	7.81 ± 0.1	7.51 ± 0.0
Algae								
Treatment	17.3 ± 0.8	16.5 ± 0.3	35.0 ± 0.6	34.9 ± 0.4	7.19 ± 0.3	4.70 ± 0.5	7.78 ± 0.1	7.66 ± 0.1
Control	17.2 ± 0.8	16.6 ± 0.3	35.0 ± 0.6	34.9 ± 0.4	7.17 ± 0.3	5.55 ± 0.4	7.80 ± 0.1	7.62 ± 0.1
(b) 200 kPa (24 h)								
Mussels (Large)								
Treatment	14.9 ± 0.0	15.8 ± 0.0	35.6 ± 0.0	35.5 ± 0.0	7.88 ± 0.0	1.41 ± 0.4	8.07 ± 0.0	7.02 ± 0.0
Control	15.4 ± 0.0	15.7 ± 0.0	36.3 ± 0.2	36.2 ± 0.2	7.48 ± 0.1	1.16 ± 0.0	7.80 ± 0.1	7.12 ± 0.0
Mussels (Small)								
Treatment	14.9 ± 0.0	15.7 ± 0.0	35.7 ± 0.0	35.5 ± 0.0	7.87 ± 0.0	1.58 ± 0.2	8.05 ± 0.0	7.39 ± 0.0
Control	15.3 ± 0.0	15.9 ± 0.0	36.6 ± 0.0	36.5 ± 0.0	7.57 ± 0.0	1.96 ± 0.1	7.72 ± 0	7.28 ± 0.0
Settlement plates								
Treatment	14.9 ± 0.0	15.7 ± 0.0	35.7 ± 0.0	35.5 ± 0.0	7.87 ± 0.0	1.32 ± 0.4	8.05 ± 0.0	7.24 ± 0.1
Control	15.3 ± 0.0	15.9 ± 0.0	36.6 ± 0.0	36.5 ± 0.0	7.57 ± 0.0	1.96 ± 0.1	7.72 ± 0	7.28 ± 0.0
Algae								
Treatment	14.9 ± 0.0	15.8 ± 0.0	35.6 ± 0.0	35.5 ± 0.0	7.88 ± 0.0	1.41 ± 0.4	8.07 ± 0.0	7.02 ± 0.0
Control	15.4 ± 0.0	15.7 ± 0.0	36.3 ± 0.2	36.2 ± 0.2	7.48 ± 0.1	1.16 ± 0.0	7.80 ± 0.1	7.12 ± 0.0
(c) 130 kPa (48 h)								
Mussels (Large)								
Treatment	15.1 ± 0.2	15.2 ± 0.0	29.1 ± 3.0	29.5 ± 3.0	8.51 ± 0.1	0.98 ± 0.4	8.05 ± 0.0	6.96 ± 0.0
Control	15.4 ± 0.4	15.0 ± 0.0	30.7 ± 2.3	31.0 ± 2.2	7.01 ± 0.8	1.04 ± 0.2	7.65 ± 0.1	6.97 ± 0.0
Mussels (Small)								
Treatment	14.8 ± 0.0	15.3 ± 0.0	29.1 ± 3.0	29.2 ± 3.0	8.63 ± 0.0	0.82 ± 0.3	8.05 ± 0	7.17 ± 0.0
Control	15.4 ± 0.0	15.1 ± 0.0	29.2 ± 3.0	29.4 ± 3.0	8.33 ± 0.0	2.29 ± 0.5	8.05 ± 0.0	7.30 ± 0.0
Settlement plates								
Treatment	15.1 ± 0.2	15.2 ± 0.0	29.1 ± 3.0	29.5 ± 3.0	8.51 ± 0.1	0.98 ± 0.4	8.05 ± 0.0	6.96 ± 0.0
Control	15.4 ± 0.4	15.0 ± 0.0	30.7 ± 2.3	31.0 ± 2.2	7.01 ± 0.8	1.04 ± 0.2	7.65 ± 0.1	6.97 ± 0.0
Algae								
Treatment	14.8 ± 0.0	15.3 ± 0.0	29.1 ± 3.0	29.2 ± 3.0	8.63 ± 0.0	0.82 ± 0.3	8.05 ± 0	7.17 ± 0.0
Control	15.4 ± 0.0	15.1 ± 0.0	29.2 ± 3.0	29.4 ± 3.0	8.33 ± 0.0	2.29 ± 0.5	8.05 ± 0.0	7.30 ± 0.0

Observed percentage decreases in pH measured in experimental units over the course of each trial exposure period (i.e. 24 or 48 h) are presented for each taxon in Figure 7. Similarly to DO, percentage decrease in pH was less in Trial 1 (130 kPa, 24 h) relative to Trials 2 (200 kPa, 24 h) and 3 (130 kPa, 48 h). In Trial 1, the percentage decrease in pH recorded in corresponding treatment and control units was similar; however, during Trials 2 and 3 the percentage decrease in pH in control bags was typically less than that observed in pressure treatment bags (Figure 7).

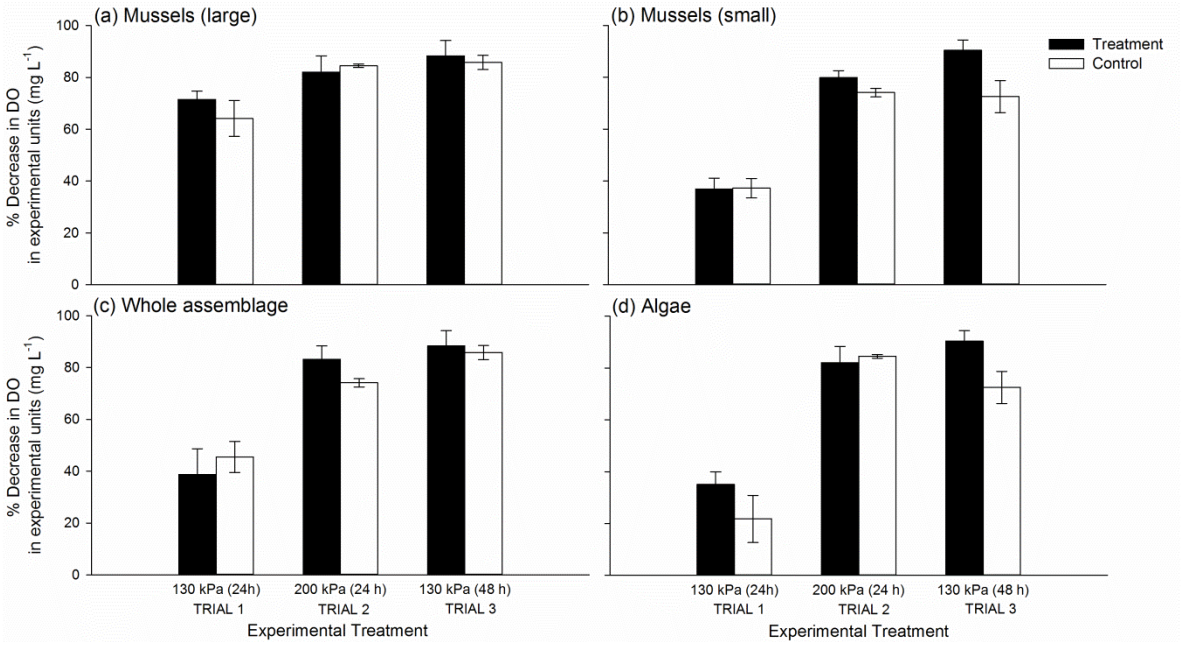

Figure 6. Plots showing the percentage decrease in dissolved oxygen (DO) recorded in experimental treatment unit bags for (a) large mussels, (b) small mussels, (c) whole assemblages, and (d) algae, over the duration of each trial exposure period (i.e. 24 or 48 h). Values represent means ±1SE (n= 3).

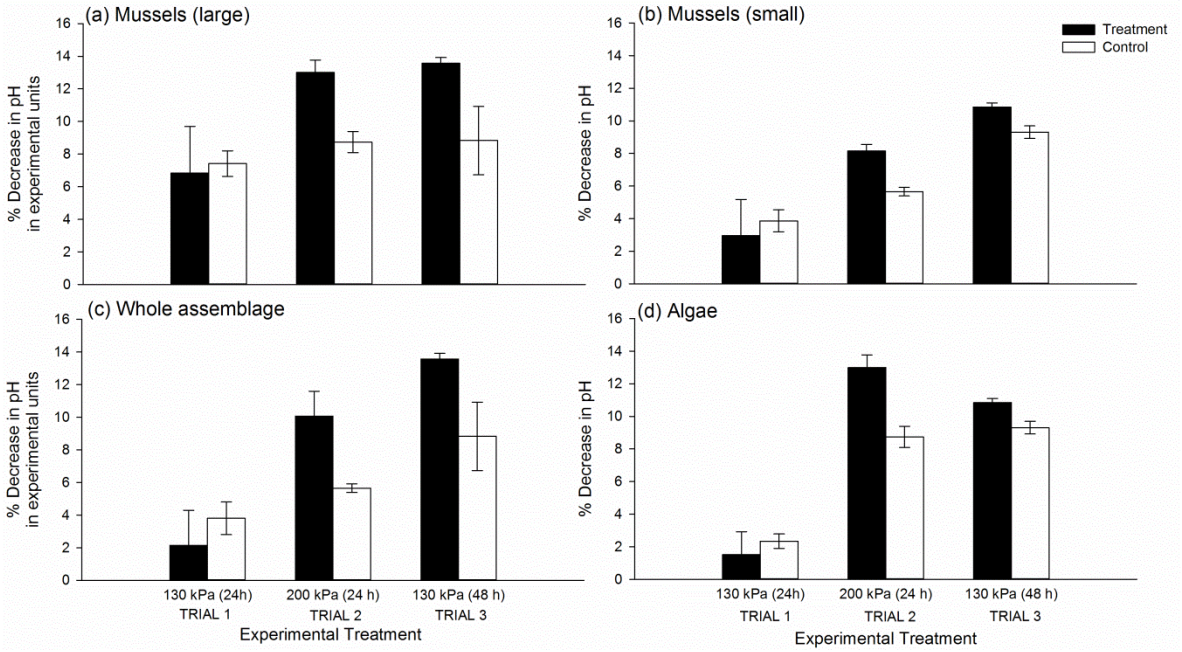

Figure 7. Plots showing the percentage decrease in pH recorded in experimental treatment unit bags for (a) large mussels, (b) small mussels, (c) whole assemblages, and (d) algae, over the duration of each trial exposure period (i.e. 24 or 48 h). Values represent means ±1SE (n= 3).

3.2 Survivorship of taxa

The survival of taxa subjected to experimental water pressure regimes are presented in Table 3. In general, large and small mussels recorded high percentage survival in treatment and control across all pressure regimes. There was a significant difference in large mussel survival among pressure profiles ($F_{(2,12)}$ = 10.11, p = 0.003; Table 3a) due decreased survival in Trial 3 (67 – 77%) relative to Trials 1 and 2 (97 – 100%) (Tukey's < 0.007; Figure 8a). For small mussels, a significant pressure profile x treatment group interaction was observed ($F_{(2,12)}$ = 138.89, p = 0.026), as a result of decreased survival in Trial 3 control group individuals (77%) relative to other treatment groups (93 – 100% survival) (Table 3b, Figure 8b).

Similarly to mussels, survival of barnacles in both treatment and control groups was typically high across all pressure profiles tested. Percentage survival of barnacles exposed to pressures of 130 kPa (24 h), 200 kPa (24 h) and 130 kPa (48 h) was 96.1, 84.7 and 74.8%, respectively, while survival of control barnacles for each of these pressure profiles was 97.7, 89.7 and 84.7%, respectively (Table 3c, Figure 8c). Analysis showed significant difference in survival of barnacles among pressure profiles ($F_{(2,12)}$ = 7.34, p = 0.008; Table 3c), which was driven by decreased survival in treatment and control barnacles from Trial 3 relative to Trial 1 (Tukey's = 0.006; Figure 8c). Survival of barnacles in Trial 2 treatment and control groups was not significantly different to survival of individuals in either Trial 1 or Trial 3 (Figure 8c).

The percentage survival of all organisms present on settlement plates was similarly high for pressure treatment and control groups in Trials 1 (treatment and control = 93.8%) and 2 (treatment = 88.8%, control = 87.7%) (Figure 8d). In contrast, survival of organisms present on

settlement plates in Trial 3 was extremely low, at 12.0 and 17.6% for pressure treatment and control group, respectively (Figure 8d). Not surprisingly, the significant difference observed among pressure profiles ($F_{(2,12)}$ = 103.55, $p < 0.001$; Table 3d) was a result of this decreased survival in Trial 3 taxa relative to Trials 1 and 2 (Tukey's < 0.001; Figure 8d).

Table 3. Survival of taxa in different treatment groups (pressure treatment and control) following exposure to different pressure treatment profiles (130 kPa [24 h], 200 kPa [24 h], 130 kPa [48 h]). Values in parenthesis indicate total number of individuals tested in each treatment group across three replicate pressure runs.

Taxa	% Survival of taxa following treatment (n)					
(PHYLUM, Species)	TRIAL 1: 130 kPa (24 h)		TRIAL 2: 200 kPa (24 h)		TRIAL 3: 130 kPa (48 h)	
	Treatment	Control	Treatment	Control	Treatment	Control
MOLLUSCA						
Mytilus galloprovincialis (Large)	100 (30)	100 (30)	96.7 (30)	100 (29)	76.7 (30)	66.7 (30)
Mytilus galloprovincialis (Small)	100 (30)	100 (30)	100 (29)	100 (29)	93.3 (30)	76.7 (30)
ARTHROPODA						
Balanus trigonus	96.1 (57)	97.7 (85)	84.7 (55)	89.7 (47)	74.8 (56)	84.7 (45)
CHORDATA						
Styella clava	91.7 (12)	100 (8)	100 (3)	100 (5)	16.7 (7)	0 (6)
Styella plicata	-	100 (1)	100 (1)	100 (3)	33.3 (3)	0 (3)
Microcosmus squamiger	83.3 (5)	88.8 (8)	88.9 (10)	55.6 (11)	0 (10)	0 (11)
Ciona intestinalis	-	100 (1)	100 (1)	100 (1)	-	-
Ascidiella aspersa	100 (2)	-	100 (1)	-	-	-
Botrylloides leachii	100 (3)	100 (3)	75 (3)	100 (2)	0 (4)	0 (1)
Didemnidae	-	-	100 (1)	50 (2)	-	-
ECTOPROCTA						
Bugula neritina	50 (4)	50 (2)	100 (10)	66.7 (7)	75 (3)	100 (2)
Bugula flabellata	83.3 (7)	50 (2)	100 (1)	100 (1)		100 (2)
Bugula avicularia	100 (3)	100 (3)	100 (8)	-	-	0 (3)
Tricellaria occidentalis	100 (6)	100 (14)	100 (4)	100 (10)	0 (9)	0 (5)
Watersipora subtorquata	100 (2)	100 (1)	100 (1)	100 (2)	100 (1)	100 (3)
PORIFERA						
Sycon sp.	95.2 (13)	66.7 (17)	60 (13)	75 (8)	0 (7)	0 (6)

Table 4. Analysis of variance (ANOVA) of the percentage survival of (a) large mussels, (b) small mussels, (c) barnacles, (d) settlement plate taxa, the (e) percentage occurrence of live tubeworms, and (f) the percentage change in frond surface area of alga exposed to three different pressure profiles (130 kPa [24 h], 200 kPa [24 h], 130 kPa [48 h]) and belonging to two different treatment groups (pressure treatment and control).

Source	df	MS	F	*p*
(a) Mussels (Large)				
Pressure Profile	2	1516.667	10.111	**0.003**
Treatment Group	1	22.222	0.148	0.707
Pressure Profile x Treatment Group	2	72.222	0.481	0.629
Error	12	150.000		
(b) Mussels (Small)				
Pressure Profile	2	450.000	16.200	**<0.001**
Treatment Group	1	138.889	5.000	**0.045**
Pressure Profile x Treatment Group	2	138.889	5.000	**0.026**
Error	12	27.778		
(c) Barnacles				
Pressure Profile	2	444.191	7.338	**0.008**
Treatment Group	1	137.517	2.272	0.158
Pressure Profile x Treatment Group	2	26.275	0.434	0.658
Error	12	60.537		
(d) Settlement Plate Taxa				
Pressure Profile	2	11682.662	103.554	**<0.001**
Treatment Group	1	9.056	0.080	0.782
Pressure Profile x Treatment Group	2	19.717	0.175	0.842
Error	12	112.817		
(e) Calcareous Tubeworms				
Pressure Profile	2	668.056	4.454	**0.036**
Treatment Group	1	138.889	0.926	0.355
Pressure Profile x Treatment Group	2	293.056	1.954	0.184
Error	12	150.000		
(f) Algal Frond Surface Area *				
Pressure Profile	1	1531.026	11.391	**0.010**
Treatment Group	1	68.319	0.508	0.496
Pressure Profile x Treatment Group	1	109.384	0.814	0.393
Error	8	134.408		

p-values in bold indicate significant differences at a=0.050

* Due to the mass mortality of algae from Trial 3 (and therefore insufficient numbers for analysis), statistical analysis of changes to frond surface area was limited to Trials 1 and 2

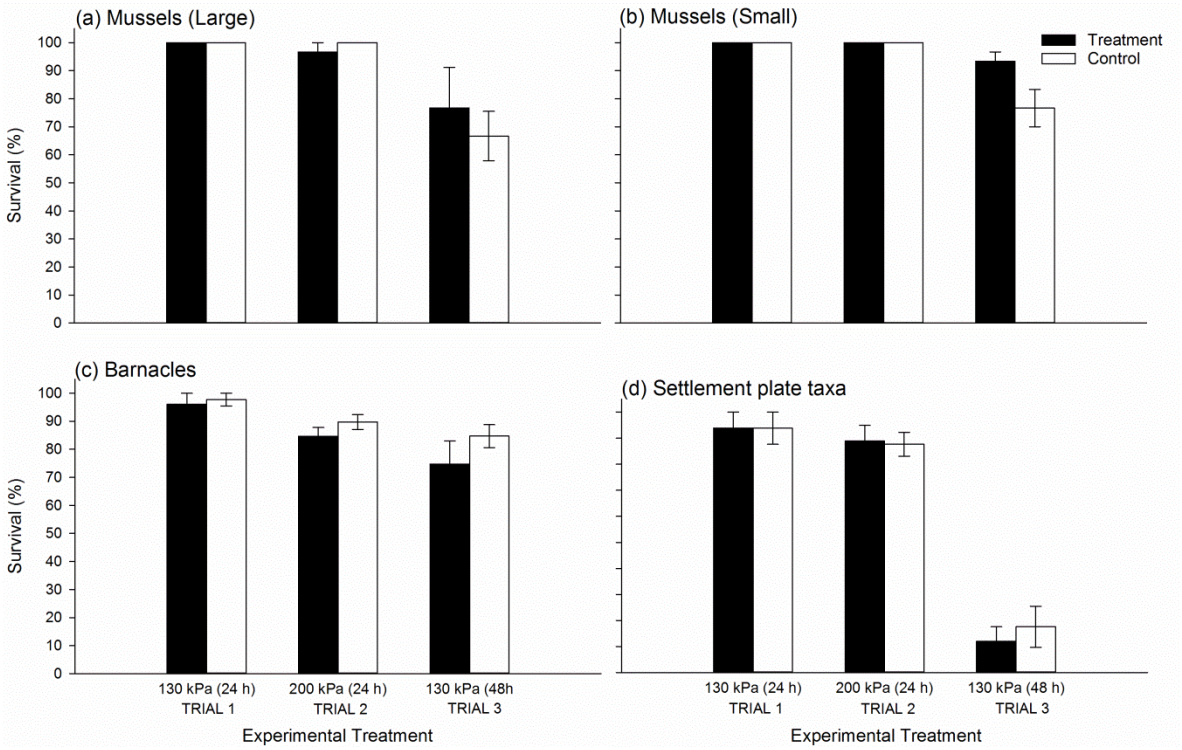

Figure 8. Survival of (a) large mussels, (b) small mussels, (c) barnacles, and (d) all settlement plate taxa, in different treatment groups (pressure treatment and control) following exposure to three pressure treatment profiles (130 kPa [24 h], 200 kPa [24 h], 130 kPa [48 h]). Values represent means ±1SE (n= 3)

The comparative presence of live worms found in 20 randomly selected tube casings from pressure treatment and control groups was used as a proxy for determining post-treatment survival of calcareous tubeworms. The mean percentage occurrence of live tubeworms in treatment and control groups was very similar for Trial 1 (treatment = 68.3%, control = 70%) and Trial 2 (treatment = 51.6%, control = 55.0%) (Figure 9). However, in Trial 3 there was a markedly greater occurrence of live worms in the treatment group (60.0%) compared to the control group (38.3%) (Figure 9). This was the major driver of the statistical difference observed among pressure profiles ($F_{(2,12)}$ = 4.54, p = 0.036; Table 3e), with the occurrence of live worms in Trial 3 samples being significantly less than those in Trial 1 samples (Tukey's = 0.038; Figure 9).

Frond surface area was used as an indicator of pressure treatment impacts on alga. Algae from Trial 1 displayed growth over the seven days post-treatment, with pressure treatment and control group plants increasing their mean frond surface areas by 3.9 and 5.1%, respectively (Figure 10). In contrast, mean frond surface area of Trial 2 plants decreased by 12.7 and 13.5% for with pressure treatment and control group plants, respectively. In Trial 3, only two control group plants survived to seven days post-treatment, and these recorded a mean decreased in surface area of 81.9% (Figure 10). Due to the mass mortality of algae from Trial 3, statistical analysis of changes to frond surface area was limited to Trials 1 and 2, with a significant difference between Trial 1 and 2 evident ($F_{(1,8)}$ = 11.39, p = 0.010; Table 3f).

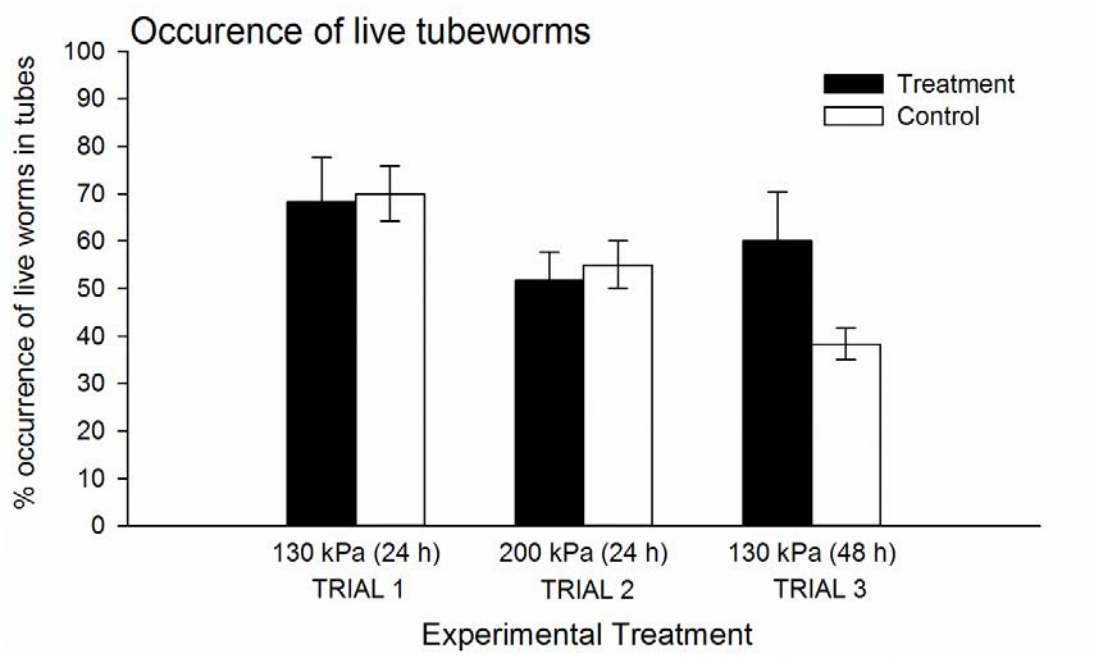

Figure 9. Percentage occurrence of live tubeworms found within twenty randomly sampled tubes from settlement plates belonging to different treatment groups (pressure treatment and control) following exposure to three pressure treatment profiles (130 kPa [24 h], 200 kPa [24 h], 130 kPa [48 h]). Values represent means ±1SE (n= 3)

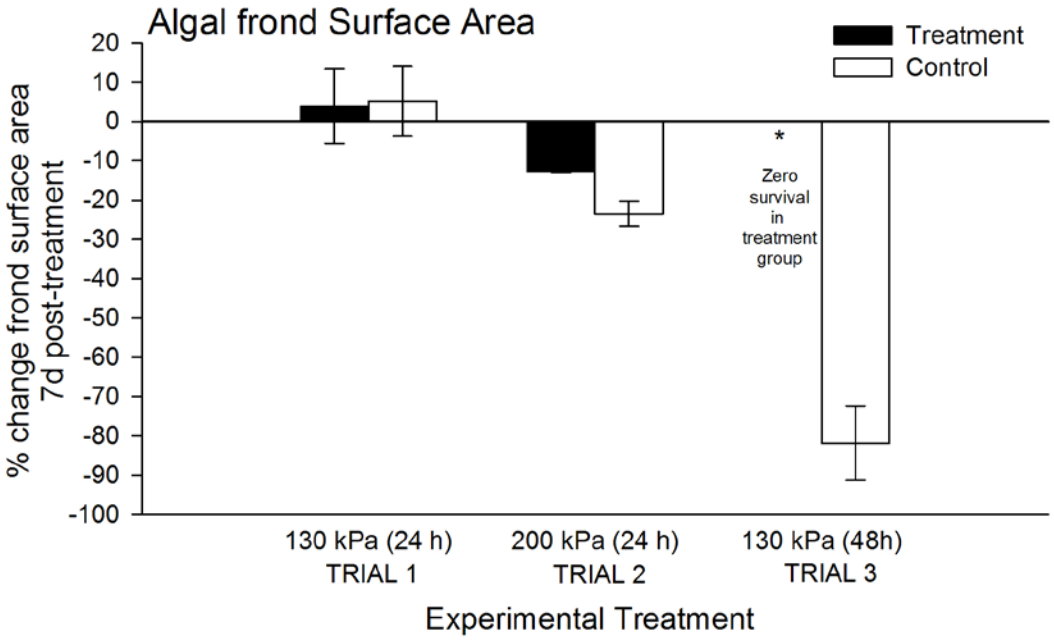

Figure 10. Percentage change in frond surface area for Undaria pinnatifida plants belonging to different treatment groups (pressure treatment and control) following exposure to three pressure treatment profiles (130 kPa [24 h], 200 kPa [24 h], 130 kPa [48 h]). Values represent means ±1SE (n= 3). Note, there was zero survival in the Trial 3 treatment group.

4. Discussion

The results of this study showed little effect of water pressure on the survival of common fouling. It is believed that the majority of adverse impacts on the organisms observed in this study were as a result of exposure to declining water quality (potentially in conjunction with exposure to water pressure) rather than the exposure to high water pressures alone. No significant difference in survival was recorded between Trial 1 and 2 even though water pressure was increased from 130 kPa to 200 kPa; however, significance differences were noted between Trials 1 and 3 which were conducted at the same pressure, but for different durations of exposure. During the extended exposure period in Trial 3, DO and pH showed marked decreases from initial starting values, indicating a decrease in water quality during the trial period. Survival was evident across all treatment regimes, although the 48 hour treatment (Trial 3: 130 kPa, 48 hours, 4 pressure cycles) showed decreased survival levels of taxa when compared to both 24 hour treatments (Trial 1: 130 kPa, 24 hours, 2 pressure cycles and Trial 2: 200 kPa, 24 hours, 2 pressure cycles). This is likely a result of the impact of extended exposure to declining water quality, rather than extended exposure to water pressure.

4.1 Physical parameters

As stated previously in this report, water temperature across the experimental units and controls varied little over the experimental period and is not considered a significant factor in the survival of the fouling taxa in this study. Recorded water temperatures (min = 14.8 ± 0.0 °C, max = 17.3 ± 0.8 °C) were within normal seasonal environmental ranges expected to be experienced by Hobsons Bay fouling taxa examined during the study (Environment Protection Authority Victoria, 2012). Salinity varied across the three different trials; however, showed little intra-trial variation, with recorded values showing virtually no change over the trial period. Trial 3 had salinities approximately 5 - 7 ‰ lower that the salinity recorded for Trials 1 and 2. Temporarily lowered salinities are generally well tolerated by the fouling taxa used in this experiment, as evidenced by their growth at the DSTO Marine Coatings and Corrosion Test Facility, Williamstown, which is subject to periodic freshwater input from the adjacent Yarra River. The variation in salinity recorded during the trial is not considered to have significantly impacted survival of organisms in Trial 3.

Other physical parameters measured, such as pH and DO, showed significant variation across the samples and may have adversely influenced the survival of the organisms.

All test units showed a decrease in DO over the trial period, as expected, with decreases in treatment units and control units being of a similar magnitude across all trials. The reduction in DO over the trial period was significantly less in Trial 1 than in Trials 2 or 3. Trials 2 and 3 showed similar decreases in DO at the end of the trial; however, survival of taxa was greater in Trial 2 than Trial 3 suggesting that low DO levels were not the only factor influencing survival. Increased survival in Trial 2 may be attributed to a shorter exposure time to the low DO conditions, rather than any further decrease in DO over the extended trial period of Trial 3. Similarly, decreases in pH were observed during all trials, with Trials 2 and 3 showing a significantly greater decrease in pH than that recorded during Trial 1. The recorded decrease in Trial 1 was similar across treatment and control units; however, the control units in Trials 2 and

3 did not show as large a decrease in pH as the treatment units. The recorded difference may be a result of increased pressure on the treatment units relative to the control units. This enables increased amounts of CO_2 to be dissolved, resulting in decreased pH. It was likely that Trial 3 had increased levels of CO_2 dissolved as a result of the release of decomposition products from dying taxa, or as by-products of organism aerobic respiration building up over the extended trial period.

Given Trials 1 and 2 were of 24 hours duration, whilst Trial 3 was twice as long at 48 hours duration, it is suggested that the decreased rates of survival in Trial 3 may have been influenced by declining water quality over the increased treatment period, rather than extended exposure to high water pressure. It was observed when handling the test units at the conclusion of Trial 3 that the water in the test bags was very cloudy and had a distinctive odour, suggestive of decaying marine organisms. Mussel spawning had been observed while the organisms were being acclimatised to the local ambient conditions in a holding tank, and it is thought that this may also have adversely affected the water quality to an extent that survival was compromised. This conclusion is supported by the fact that small and large mussels and barnacles all recorded <70% survival despite pressure treatment, with some pressure treatments (small mussels and barnacles) recording greater survival relative to controls (Figure 8).

4.2 Survivorship of taxa

Trials 1 and 2 recorded high levels of survival across all taxa, in contrast to Trial 3 which recorded a significant decrease, particularly in soft bodied organisms on the settlement plates and algae. The only exceptions to this were for (i) barnacle survival, for which Trial 3 results were significantly different to those of Trial 1, but not Trial 2, and (ii) algae survival, which was not able to be analysed for Trial 3 due to insufficient replication caused by mass mortality and extensive frond degradation in surviving individuals.

Hard bodied organisms such as the barnacles and large and small mussels generally showed greater survival compared to the soft bodied species. This may be due to their ability to effectively ‘isolate’ themselves from adverse local conditions by closing their shell halves or operculum and suspending feeding and respiratory activities. In contrast, soft bodied organisms, such as those forming the majority of the mixed community assemblage on the settlement plates, and algae recorded lower levels of survival than the hard bodied species tested. This may in part be due to the relatively higher levels of exposure to poor local water quality, increased pressure and, in the case of the algae, the zero light conditions present in the test vessels. It is worth noting that it would be unlikely to find algae in a low light niche or free flood space on a submarine, but would be likely to be found on the external hull, particularly toward the waterline and would still be exposed to changing water pressure during operations.

Tubeworm survival was significantly reduced in Trial 3 compared to Trial 1. However, the difference was driven by reduced survival recorded in the control group rather than mortality in the treatment group. This may be a factor of the assessment protocol that required a random selection of 20 tubes for survival assessment. The nature of the assessment may have resulted in a low percentage of surviving individuals being randomly selected and hence influenced the survival results recorded. In future, increasing the sample size of worm tubes examined may overcome some of this variability.

Generally, whilst mortality increased in treatments compared to controls and with increasing exposure time, significant survival was still evident across all treatment profiles and the mortality that occurred is likely not significantly attributable to water pressure changes, but rather a combination of factors influencing local water quality.

4.3 Limitations of the study

The present study attempted to replicate the changes in water pressure that may be experienced by biofouling taxa in the free flood spaces of a submarine using a series of closed treatment systems, namely (i) a contained pressure vessel of fixed volume and temperature, and (ii) an enclosed experimental test unit (i.e. 'a bag') of small fixed volume. While these shortcomings in the experimental methodology were considered acceptable for the purposes of this study, it should be noted that they are not reflective of the conditions experienced by biofouling in the free flood spaces of a submarine. Most submarine niches and free flood spaces would likely receive adequate free exchange of water such that poor local water quality conditions (caused by respiration and waste excretion) would not develop during the course of a voyage. However, these observations from the present study do highlight the potential utility of encapsulation and periodic water restriction as a potential control strategy for fouling in difficult to reach free flood spaces and niche areas. Additionally, real world water pressure changes associated with a submarine diving and/or surfacing would also have corresponding changes in water temperature (e.g. decreasing water temperatures associated with depth). The possibility exists that a combination of rapid pressure and temperature changes (analogous to conditions experience when a submarine dives to depth) may act synergistically, compounding the impact they either of these parameters have on biofouling survival when considered in isolation. This was not tested at this time.

5. Recommendations

Based on the results of the current study, a reliance on the effects of water pressure change during operational diving and surfacing manoeuvres is not recommended as a singular niche area fouling mitigation strategy for submarines. Whilst water quality changes are thought to have adversely impacted on survival of the fouling taxa treated, significant survival of the fouling taxa was still recorded, thus showing water pressure changes alone to be insufficient as a control mechanism.

If the experimental protocol is repeated, it is recommended that water is changed after 24 hours of testing, to ensure that impacts attributable to pressure changes are not confounded by impacts due to poor local water quality. Poor water quality may also be improved by having the test units contained in larger volumes of water, with the placement of only one species into a test unit. Organisms such as mussels adversely impact water quality which may affect less robust organisms contained in the same bag. It is further recommended that any future studies also consider the combined effect of oscillating temperature in association with pressure changes, in order to better reflect the 'real world' parameters of diving to depth in an ocean environment.

6. Acknowledgements

We thank Michael O'Reilly and Jeff Seers (MD, DSTO) for their assistance demonstrating the operation and maintenance requirements of the pressure vessel used in the test protocol.

7. References

ASA (2007) *Assessment of introduced marine pest risks associated with niche areas in commercial shipping: final report*. Melbourne, Victoria, Australian Shipowners Association

Coutts, A. D. M. and Dodgshun, T. J. (2007) The nature and extent of organisms in vessel sea-chests: A protected mechanism for marine bioinvasions. *Marine Pollution Bulletin* **54** (7) 875-886

Environment Protection Authority Victoria (2012) *Baywide Water Quality Monitoring Program Milestone Report No.9 Port Phillip Bay Water Quality: November 2007 - December 2011* Environment Protection Authority Victoria, Editor. Melbourne. Environment Protection Authority Victoria.

IMO (2011) *Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species. Annex 26. Resolution MEPC.207(62)*. International Maritime Organisation

Polglaze, J. (2009) *Report (for Consultation) Project SEA1000 Future Submarine: Initial Environmental Compliance Review*. 42907257-2163/R1438/M&C3168/01, Perth, URS Australia Pty Ltd

URS (2006) *Biofouling survey of RAN ships and submarines: Part 1 - Summary report*. URS Australia Pty Ltd, Perth, Australia. Prepared for Department of Defence

DEFENCE SCIENCE AND TECHNOLOGY ORGANISATION
DOCUMENT CONTROL DATA

1. PRIVACY MARKING/CAVEAT (OF DOCUMENT)

1. TITLE Biosecurity Management of Submarine Niche Areas: the Effect of Water Pressure on Biofouling Survival	3. SECURITY CLASSIFICATION (FOR UNCLASSIFIED REPORTS THAT ARE LIMITED RELEASE USE (L) NEXT TO DOCUMENT CLASSIFICATION) Document (U) Title (U) Abstract (U)
4. AUTHOR(S) Clare Grandison and Richard Piola	5. CORPORATE AUTHOR DSTO Defence Science and Technology Organisation 506 Lorimer St Fishermans Bend Victoria 3207 Australia

6a. DSTO NUMBER	6b. AR NUMBER	6c. TYPE OF REPORT	7. DOCUMENT DATE
DSTO-TR-2930	AR-015-833	Technical Report	January 2014

8. FILE NUMBER	9. TASK NUMBER	10. TASK SPONSOR	11. NO. OF PAGES	12. NO. OF REFERENCES
2013/1204957/1	07/359	DDNEP	20	6

13. DSTO Publications Repository	14. RELEASE AUTHORITY
http://dspace.dsto.defence.gov.au/dspace/	Chief, Maritime Division

15. SECONDARY RELEASE STATEMENT OF THIS DOCUMENT

Approved for public release

OVERSEAS ENQUIRIES OUTSIDE STATED LIMITATIONS SHOULD BE REFERRED THROUGH DOCUMENT EXCHANGE, PO BOX 1500, EDINBURGH, SA 5111

16. DELIBERATE ANNOUNCEMENT

No limitations

17. CITATION IN OTHER DOCUMENTS No

18. DSTO RESEARCH LIBRARY THESAURUS

Biofouling, Fouling Organisms, Marine Biosecurity, Niche Area, Submarine

19. ABSTRACT

This study showed that exposure to pressure change has a minimal impact on the survival of common biofouling taxa; however, fouling taxa survival may be adversely impacted by declining local water quality. Survival was evident across all water pressure treatment regimes, although the 48 hour treatment (Trial 3: 130 kPa, 48 hours, 4 pressure cycles) resulted in decreased survival compared to both 24 hour treatments (Trial 1: 130 kPa, 24 hours, 2 pressure cycles and Trial 2: 200 kPa, 24 hours, 2 pressure cycles). Whilst the study resulted in some mortality of the fouling taxa, significant survival of the fouling taxa was still recorded, thus showing water pressure change to be insufficient as a control mechanism. Based on the results of the current study, a sole reliance on the effects of water pressure change during operational diving and surfacing manoeuvres is not recommended as a niche area fouling mitigation strategy for submarines and consideration of other mitigation strategies is required to ensure biofouling is controlled to reduce operational impacts and biosecurity risks.